# Degradation of foot-and-mouth disease virus during composting of infected pig carcasses

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## Abstract

The objective of this study was to investigate the inactivation and degradation of foot-and-mouth disease (FMD) virus during composting of infected pig carcasses as measured by virus isolation in tissue culture and by real-time reverse transcriptase polymerase chain reaction (RRT-PCR). Three FMD-infected pig carcasses were composted in a mixture of chicken manure and wood shavings in a biocontainment level 3 facility. Compost temperatures had reached 50°C and 70°C by days 10 and 19, respectively. Under these conditions, FMD virus was inactivated in specimens in compost by day 10 and the viral RNA was degraded in skin and internal organ tissues by day 21. In comparison, at ambient temperatures close to 20°C, FMD virus survived to day 10 in the skin tissue specimen from the pig that had the highest initial level of viral RNA in its tissues and the viral RNA persisted to day 21. Similarly, beta-actin mRNA, tested as a PCR control, persisted to day 21 in specimens held at ambient temperatures, but it was degraded in the remnants of tissues recovered from compost on day 21. Results from this study provide evidence that composting could be used for safe disposal of pig carcasses infected with FMD virus.

## *Résumé*

*L'objectif du présent projet était d'étudier l'inactivation et la dégradation du virus de la fièvre aphteuse (FMD) durant le compostage de carcasses de porc infectées tel que mesuré par isolement viral en culture tissulaire et par réaction d'amplification en chaîne réverse par la polymérase en temps réel (RRT-PCR). Trois carcasses de porc infectées par le FMD ont été compostées dans un mélange de fumier de poulet et de copeaux de bois dans des installations de niveau de confinement biologique 3. La température du compost a atteint 50°C et 70°C après respectivement 10 et 19 jours. Dans ces conditions, le virus FMD a été inactivé dans les spécimens dans le compost après 10 jours et l'ARN viral a été dégradé dans la peau et les organes internes au jour 21. En comparaison, à température ambiante près de 20°C, le virus FMD a survécu jusqu'au jour 10 dans l'échantillon de peau du porc qui avait le plus haut niveau initial d'ARN viral dans ses tissus et l'ARN viral a persisté jusqu'au jour 21. De manière similaire, l'ARN de la bêta-actine, utilisé comme témoin du PCR, a persisté jusqu'au jour 21 dans les échantillons gardés à température ambiante, mais il était dégradé dans les restes de tissus récupérés du compost au jour 21. Les résultats de la présente étude fournissent des évidences que le compostage pourrait être utilisé pour l'élimination sécuritaire de carcasses de porc infectées avec le virus FMD.*

*(Traduit par Docteur Serge Messier)*

## Introduction

Foot-and-mouth disease (FMD) virus is a member of the *Aphthovirus* genus within the *Picornaviridae* family. Foot-and-mouth disease virus particles lack a lipid envelop, and their infectivity is insensitive to organic solvents, but labile at pH values of $< 6.0$ (1). The virus causes a highly contagious vesicular disease of domesticated and wild ruminants and pigs. Other susceptible wild species include hedgehogs, armadillos, nutrias, elephants, capybaras, rats, and mice. Ruminants are usually infected via inhalation of infectious droplets exhaled by infected animals (2–4), and pigs are often infected by consumption of unprocessed contaminated animal products (5). In addition, infection may be spread by direct contact with virus on fomites that are contaminated by animal secretions and excretions, such as saliva, milk, urine, and feces (6). Clinical signs of FMD consist of a febrile response, excessive salivation, and development of vesicular lesions at predilection sites in the oral cavity and on tongue, muzzle, nares, teats, coronary bands, and interdigital spaces (5). Most infected animals eventually recover but often develop sequelae including sterility, abortion, excessive weight loss, significant loss in milk production, heart damage, lameness, and general poor condition.

As FMD can lead to significant economic consequences, many countries implement a "stamping out" policy established by the Office international des épizooties (OIE), where entire herds that contain infected animals are depopulated (7). In the 2001 FMD outbreak in the United Kingdom, more than 6 500 000 animals were slaughtered, and the various methods for disposal of carcasses

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included on-farm burial, on-farm burning, commercial incineration, rendering, licensed landfill, and mass burial in engineered sites (8). However, difficulties were encountered in implementing disposal procedures. On-farm burial was restricted by legislation to protect groundwater supplies. Pyre burning was limited by public concerns related to smoke and other emissions, and both rendering facilities and licensed landfill sites were insufficient to meet the demand. There is a need for environmentally sound alternatives for the safe disposal of animal carcasses in the event of disease outbreaks or major natural disasters. Biosecurity agencies in Canada, the USA, Australia, and New Zealand have recognized the potential benefits of using composting for routine and emergency management of mortalities (9). On-site composting was successfully used for the safe disposal of more than 500 000 chicken carcasses during the 2004 avian influenza outbreak in British Columbia (10). The success of this operation suggested that composting strategies could be developed for the safe disposal of animal carcasses infected with other viruses, including FMD virus. Thus, the objective of the present study was to provide information on the fate of FMD virus in pig carcasses during composting using both real-time reverse transcriptase polymerase chain reaction (RRT-PCR) and tissue culture methods to measure viral presence and survival.

# Materials and methods

## Compost construction

Chicken manure for the compost was obtained from a cage layer operation at the University of Manitoba, Winnipeg, Manitoba and was mixed with wood shavings on a 1:1 dry weight basis. Sufficient water was added to adjust the moisture content of the mixture to approximately 65%, as measured with an IR-35 moisture analyzer (Denver Instrument, Denver, Colorado, USA). Mixing was done on the university premises using a feed mixer.

The composting experiment was conducted in a biosecure cubicle within a level 3 biocontainment facility at the National Centre for Foreign Animal Disease (NCFAD), Winnipeg, Manitoba. The compost bin, as illustrated in Figure 1, was constructed with Styrofoam panels and aluminium frames for the walls and floor. The interior of the bin was lined with a heavy plastic sheet, as previously described (11). To provide passive aeration, 2 pieces of perforated and flexible Big 'O' plastic drainage tubing (10 cm interior diameter, 3.5-m long; Armtec Limited, Orangeville, Ontario) were installed near the bottom of the bin with the ends projecting out above the top of the compost. The carcasses of 3 pigs that had been infected with FMD virus were buried in the compost mixture. The top of the compost was covered with a vapor barrier fabric. This was then covered with a 20-cm layer of wood shavings that served as insulation.

## Infection of pigs by FMD virus

Three 5- to 6-week-old Landrace cross pigs, each weighing 7 to 9 kg, were used for this study. Each pig received a total of $10^{6.7}$ tissue culture infectious doses ($TCID_{50}$) of a highly virulent FMD virus (serotype O UKG 11/2001) via the oral and intranasal routes and also intradermally by inoculating it onto abraded coronary bands. All pigs were maintained in a biosecure cubicle within a level 3 biocontainment facility at NCFAD. The animal care procedures for this study conformed to guidelines established by the NCFAD Animal Care Committee, and were observed by an NCFAD veterinarian. The 3 pigs were euthanized at 2 or 3 d post-inoculation (dpi) and all 3 carcasses were composted within 24 h of storage at 20°C.

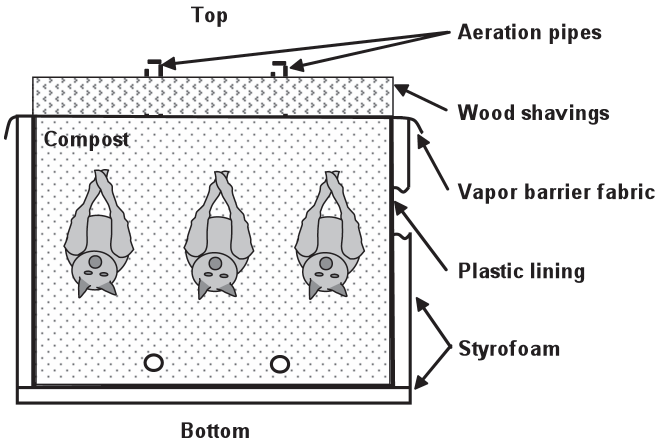


**Figure 1. Cross section of the compost bin which had internal dimensions of 120 cm (L) × 110 cm (W) × 100 cm (H). Three pig carcasses were buried horizontally about 50 cm from the bottom of the bin and were spaced about 15 to 20 cm apart. Two pieces of aeration tubing were installed in the bin in a U shape such that the bottom of the U was about 10 cm from the bottom of the bin. The two Us were about 40 cm apart and their ends extended up out of the compost.**

## Specimen preparation

Carcasses of the 3 pigs were designated as C1, C2, and C3. Postmortem examinations were performed on C1 and C2 and 2 pools of specimens were collected from each carcass. One pool included skin tissues from coronary bands, interdigital cleft, hock, and snout. The other consisted of tissues from tonsils, thyroids, prescapular lymph nodes, adrenal glands, and kidneys. Each specimen pool was divided into 5 equal-sized portions. One portion was immediately stored at −80°C to serve as a time 0 control. Two others were placed in plastic bags that were held outside of the compost bin as ambient temperature controls. Another 2 specimens were contained in nylon mesh bags that were permeable to air, moisture, and microbes. Tissues in mesh bags were buried in compost where they were surrounded by plastic netting attached to metal chains to facilitate specimen recovery (12). The skin tissues were kept beside the corresponding carcass and organ tissues were placed inside the abdominal cavity that was later closed with sutures. On days 10 and 21, one specimen of both skin and organ tissues from C1 and C2 were removed from compost and from the ambient temperature environment. The C3 carcass was not opened but following removal of small pieces of skin tissues and of the epithelial layer of the tongue to serve as time 0 controls, the carcass was buried in compost. Temperatures within and outside the compost bin were recorded with stainless steel Hobo Temp Data loggers U12-015 (Onset Computer Corporation, Bourne, Massachusetts, USA). One logger was placed inside each of the plastic nettings that surrounded specimens from C1 and C2. In C3, one logger was inserted into the mouth and another into the rectum, and both the mouth and anal openings were then closed with sutures.

## Virus extraction

To extract virus from composted and control tissues, the specimens were first homogenized using a mortar and pestle with the aid of Alumdum particles (60 mesh, Fisher Scientific, Ottawa, Ontario) in 50 mL of 10% beef extract (pH 8.0; Becton, Dickinson and Company, Oakville, Ontario). The homogenate was centrifuged at 5000 × *g* for 60 min at 4°C for separation of compost or tissue debris. The supernatant was collected and 45 mL was mixed with an equal volume of 16% polyethylene glycol 6000 (pH 7.2; Sigma, St. Louis, Missouri, USA) in 0.01 M phosphate buffered saline (PBS, pH 7.2). The mixture was incubated at 4°C overnight, followed by centrifugation at 10 000 × *g* for 90 min at 4°C to precipitate the virus. The supernatant was discarded and the pellet was suspended in 12 mL of Dulbecco's PBS (Invitrogen, Burlington, Ontario) for both the RNA extraction and virus isolation.

## Virus isolation

The above pellet suspension was treated with a cocktail of streptomycin, vancomycin, nystatin, and gentamycin as described previously (13). The treated suspension was passed through an ultra-low protein binding membrane filter with a pore size of 0.45 μm (Millipore, Nepean, Ontario). The filtrates were serially diluted in Dulbecco's PBS and then inoculated into primary lamb kidney (LK) cell cultures (14). The cell cultures were examined for cytopathic effect (CPE) after 48 h of incubation and were subjected to a freeze and thaw cycle to release virus into the culture fluids. To confirm the presence of the FMD virus in cell cultures with CPE, culture fluids were tested by RRT-PCR as described below and by double antibody sandwich ELISA (15). Rabbit anti-FMD O1 BFS 1860 sera and guinea pig anti-FMD O1 BFS 1860 sera produced at NCFAD were used as the capture antibody and the detector antibody, respectively. The culture fluids from the cell cultures without CPE were inoculated into fresh LK cell cultures for a second passage in an attempt to detect virus.

## Quantification of viral RNA

Extraction of RNA was performed using the RNeasy mini kit (Qiagen, Mississauga, Ontario) with 375 μL of culture fluid or pellet suspension that resulted from virus extraction from composted and control specimens. In separate RRT-PCR assays, FMDV-3D primers and probe were used for absolute quantification of FMD viral RNA, and beta-actin primers and probe were used to amplify endogenous beta-actin mRNA from pig tissues as a control against false-negative results (14). Both assays were performed using reagents supplied from the QuantiTect Probe RT-PCR kit (Qiagen). A 25 μL reaction volume was used, including 2 μL of RNA template for the FMD virus assay or 5 μL of RNA template for the beta-actin assay. A final concentration of 0.5 μM of each forward and reverse primer and 0.2 μM of the probe were used in the FMD virus assay, and 1.0 μM of each primer and 0.2 μM of the probe were used for the beta-actin assay. Identical cycling conditions were used for both assays with an SDS 7900HT thermocycler (Applied Biosystems, Foster City, California, USA): reverse transcription at 50°C for 30 min; activation at 95°C for 15 min; followed by 45 cycles of 95°C for 10 s and 60°C for 1 min.

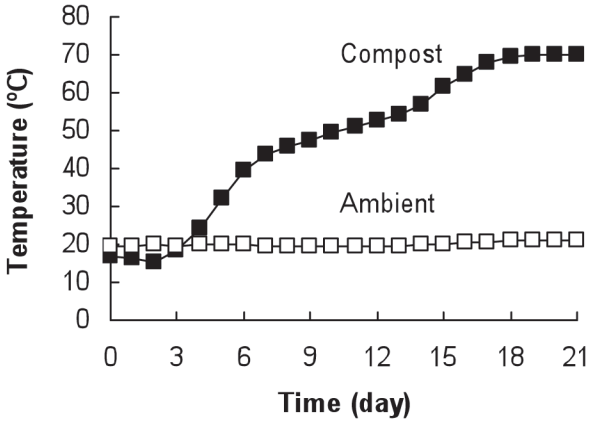


**Figure 2. Temperature and time profiles. Data are average temperatures recorded by 8 loggers in compost (dark square) and 2 loggers outside of compost (white square). Standard deviation(s) were < 2°C.**

# Results

## FMD virus infection

Within 2 or 3 d post-inoculation (dpi) with the FMD virus, all 3 pigs developed typical symptoms of disease that included severe depression, vesicles on feet, rupture and bleeding of vesicles, blanching on coronary bands, and some sloughing of the hoof shells. The pigs were euthanized within 3 dpi and their carcasses were composted.

## Compost operation

The compost experiment was conducted for 21 d to study the survival of the FMD virus in the pig carcasses. Compost temperatures reached 50°C by day 10 and 70°C by day 19 (Figure 2). When the contents of the compost were examined on day 21, the remains of the carcasses consisted of scattered remnants of skin and muscle and some bones that could be readily crushed by hand.

## The fate of FMD virus and viral RNA

Quantitative information on levels of virus in tissues was based on RRT-PCR results. Prior to composting, the concentration of FMD viral RNA was at least 2 $\log_{10}$ higher in tissues from C2 than that in tissues from C1 (Figure 3 A & B). In specimens removed from compost on day 10, the viral RNA was not detected in specimens from C1 but there were still more than 4 $\log_{10}$/g of the viral RNA in specimens from C2. No viral RNA was detected in any of the specimens removed from the compost on day 21. In comparison, for specimens held at ambient temperatures (20°C), the viral RNA was detected in the skin tissue specimen from C1 collected on day 10 and in both the skin and the organ tissue specimens from C2 collected on days 10 and 21 (Figure 3 A & B). Beta-actin mRNA was not detected in any specimen removed from the compost on day 21 but it was detected in specimens removed on day 10 and all specimens held at ambient temperatures (data not shown). To more fully study virus survival in carcasses, C3 was kept intact

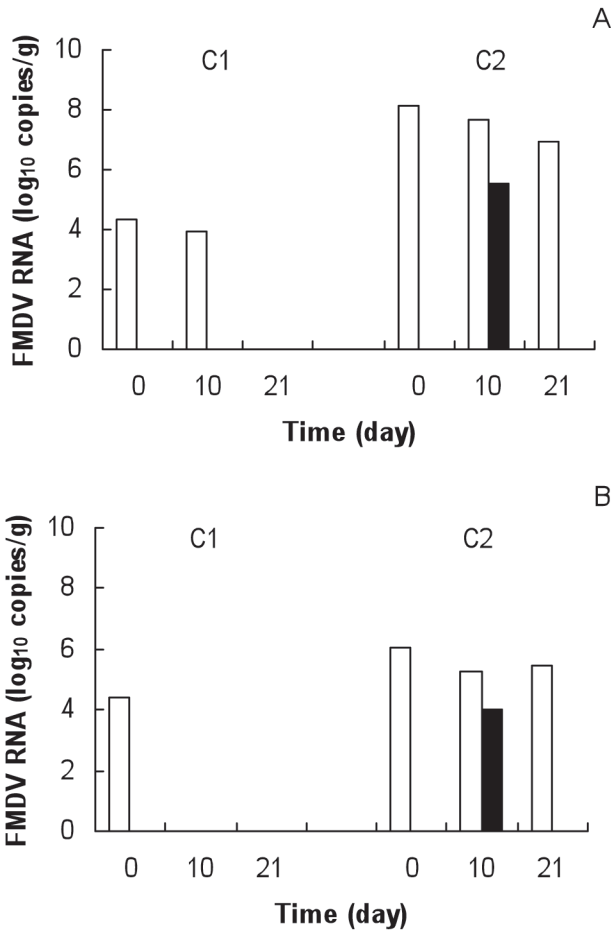


**Figure 3. The fate of FMD viral RNA in skin (A) or organ (B) tissues in 2 infected pigs (C1 and C2). White bars represent levels of viral RNA in specimens at time 0 or at ambient temperatures whereas black bars represent the levels in compost.**

except for removal of small pieces of external tissues. At time 0, the concentration of the FMD viral RNA in skin tissues from C3 was 8.2 $\log_{10}$ copies/g (data not shown). When the compost study was terminated on day 21, neither viral nor beta-actin mRNA were detected in compost material that contained remnants of skin and other tissues of C3.

Foot-and-mouth disease virus was isolated from all time 0 skin tissue and organ tissue specimens from the 3 carcasses and the findings on skin tissue specimens are reported in Table I. All specimens that were removed from compost on days 10 and 21 were negative for the virus. Among specimens held at ambient temperatures, virus was isolated on day 10 from the skin tissues of C2 but was not isolated from any of the other specimens on days 10 or 21.

## Discussion

The present study demonstrated that FMD virus in carcasses of infected pigs could be readily destroyed during composting in a plastic-lined insulated bin that was passively aerated. The temperatures in the compost reached 50°C within 10 d and approached 70°C for a short period by day 19. The feasibility of using the same

**Table I. Survival of foot-and-mouth disease virus in skin tissues that were composted or held at ambient temperatures**

| | FMD virus | | | | |
|---|---|---|---|---|---|
| | | Compost (20°C to 70°C) | | Ambient temperatures (20°C) | |
| Origin of skin tissues[a] | Time 0 | Day 10 | Day 21 | Day 10 | Day 21 |
| C1 | +[b] | – | – | – | – |
| C2 | + | – | – | + | – |
| C3 | + | NA | – | NA | NA |

[a] Skin tissues were removed from all 3 carcasses (C1, C2, and C3) prior to composting. Tissues from C1 and C2 were contained in mesh bags or plastic bags that were placed in compost or held at ambient temperatures, respectively. Composted materials collected from C3 on day 21 contained remnants of skin.

[b] Specimens were tested by the virus isolation method using primary lamb kidney cells. Cell culture fluids were tested by both ELISA and real-time RT-PCR to confirm the presence of FMD virus. NA — specimen not available.

composting methods for disposal of cattle carcasses in the event of an FMD-outbreak was evident from earlier studies where 32 animals, which were free of the virus, were composted. The temperatures in the compost reached 55°C to 65°C and during a 147-day period, all but large bones were converted to compost suitable for disposal on land (16). The heavy plastic liner in the compost bin for the present study contained the leachate, and thereby the virus, to the bin. In fact, as in earlier studies (11,12,16) very little leachate accumulated within the plastic-lined structures. Thus, this type of containment would allow composting to be conducted on farms without causing contamination of groundwater with viruses or other pollutants (17). This is important for disease control purposes since FMD virus has been shown to survive for more than 6 mo on the soil surface under snow (6). In this study, the compost mix surrounding the pig carcasses facilitated good carcass degradation and provided filtration to minimize the release of odor.

In addition to inactivating the FMD virus, the study demonstrated that the viral RNA could be degraded in tissues of infected carcasses within 21 d in compost, provided the temperatures were similar to those achieved herein. As expected, the initial virus loads varied in the tissues derived from different infected animals, and the time required for virus inactivation and viral RNA degradation appeared to be positively correlated with the initial concentrations of the virus in the infected tissues. This observation was consistent with a report on the duration of the virus in wool and in animal excretions (6). It is noteworthy that by 21 d of composting, the FMD virus was inactivated and its viral RNA degraded in the intact carcass as well as in the opened carcasses. The negative tests for FMD viral RNA in tissue specimens recovered from compost on day 10 were not due to inhibition of the assay since beta-actin mRNA was detected in all specimens on that day. As beta-actin mRNA is a component of tissues and would be much more abundant in carcasses than the viral RNA, degradation of beta-actin mRNA may serve as a good indicator for inactivation of the virus. In this study, the degradation of beta-actin mRNA by day 21 was consistent with the observation

that only remnants of tissues could be detected in the compost on that day. The findings support our earlier observations that the RNA of transgenes in bacteria and in plant tissue could be degraded during composting (11,18).

At ambient temperatures close to 20°C, the FMD virus survived for 10 d in the skin tissue that contained the highest initial concentration of viral RNA. Inactivation of the virus at such temperatures may be attributed to the decrease of pH during postmortem biochemical changes in tissues (19) and/or microbial activity during tissue decomposition (20). Degradation of the FMD viral RNA occurred more slowly at ambient temperatures than in compost, and the findings are consistent with observations on degradation of avian influenza and Newcastle disease viral RNA at ambient temperatures and in compost (12).

In conclusion, the present study provided evidence that composting strategies designed to provide a high level of biosecurity could serve as an environmentally desirable alternative for disposal of animal carcasses infected by FMD virus.

## Acknowledgments

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## References

1. Racaniello VR. Picornaviridae: The viruses and their replication. In: Knipe DM, Griffin DE, Lamb RA, et al. eds. Fields' Virology. 5th ed. Philadelphia, Pennsylvania: Lippincott Williams & Wilkins, 2007:795–838.
2. Sellers RF. Quantitative aspects of the spread of foot and mouth disease. Vet Bull 1971;41:431–439.
3. Sellers RF, Herniman KA, Donaldson AI. The effects of killing or removal of animals affected with foot-and-mouth disease on the amounts of airborne virus present in looseboxes. Br Vet J 1971;127:358–365.
4. Donaldson AI, Alexandersen S, Sørensen JH, Mikkelsen T. The relative risks of the uncontrollable (airborne) spread of foot-and-mouth disease by different species. Vet Rec 2001;148:602–604.
5. Blackwell JH. Internationalism and survival of foot-and-mouth disease virus in cattle and food products. J Dairy Sci 1980;63:1019–1030.
6. Bartley LM, Donnelly CA, Anderson RM. Review of foot-and-mouth disease virus survival in animal excretions and on fomites. Vet Rec 2002;151:667–669.
7. Office international des épizooties. International Animal Health Code, OIE, Paris, France. 2006.
8. Scudamore JM, Trevelyan GM, Tas MV, Varley EM, Hickman G. Carcass disposal: Lessons from Great Britain following the foot-and-mouth disease outbreaks of 2001. Rev Sci Tech 2002;21:775–787.
9. Wilkinson KG. The biosecurity of on-farm mortality composting. J Appl Microbiol 2007;102:609–618.
10. Spencer JL, Guan J, Rennie B. Management related diseases—Manure management and environmental contamination. Proc World Vet Poult Cong, Istanbul, Turkey, 2005:159–162.
11. Guan J, Spencer JL, Ma BL. The fate of the recombinant DNA in corn during composting. J Environ Sci Health 2005;40:463–473.
12. Guan J, Chan M, Grenier C, Wilkie DC, Brooks BW, Spencer JL. Survival of avian influenza and Newcastle disease viruses in compost and at ambient temperatures based on virus isolation and real-time reverse transcriptase PCR. Avian Dis 2009;53:26–33.
13. Guan J, Chan M, Ma B, et al. Development of methods for detection and quantification of avian influenza and Newcastle disease viruses in compost by real-time reverse transcription polymerase chain reaction and virus isolation. Poult Sci 2008;87:833–843.
14. Moniwa M, Clavijo A, Li M, Collignon B, Kitching RP. Performance of a foot-and-mouth disease virus reverse transcription-polymerase chain reaction with amplification controls between three real-time instruments. J Vet Diagn Invest 2007;19:9–20.
15. Alonso A, Martins MA, da Penha M, Gomes D, Allende R, Söndahl MS. Foot-and-mouth disease virus typing by complement fixation and enzyme-linked immunosorbent assay using monovalent and polyvalent antisera. J Vet Diagn Invest 1992;4:249–253.
16. Xu W, Reuter T, Inglis D, et al. Development of a composting system for emergency disposal of cattle carcasses and manure during an infectious disease outbreak. J Environ Qual 2009;38:437–450.
17. Harper AF, DeRouchey JM, Glanville TD, Meeker DL, Straw BF. Swine carcass disposal options for routine and catastrophic mortality. Council Agr Sci Tech 2008;39:1–16.
18. Guan J, Spencer JL, Sampath M, Devenish J. The fate of a genetically modified *Pseudomonas* strain and its transgene during the composting of poultry manure. Can J Microbiol 2004;50:415–421.
19. Panina GF, Civard A, Massirio I, Scatozza F, Baldini P, Palmia F. Survival of foot-and-mouth disease virus in sausage meat products (Italian salami). Int J Food Microbiol 1989;8:141–148.
20. Vass AA. Beyond the grave — understanding human decomposition. Microbiol Today 2001;28:190–192.